POLYPEPTIDES

CROSS-REFERENCE TO RELATED APPLICATION(S)

[0001] This patent application is a continuation of, and therefore claims priority from, U.S. patent application Ser. No. 15/717,230 entitled POLYPEPTIDES filed Sep. 27, 2017, which is a continuation of, and therefore claims priority from, International Application No. PCT/EP2016/057024 entitled POLYPEPTIDES filed Mar. 31, 2016, which claims priority from EP 15162115.8 filed Mar. 31, 2015 and EP 16152320.4 filed Jan. 21, 2016, the contents each of which are hereby incorporated herein by reference in their entireties.

FIELD OF THE INVENTION

[0002] The present invention relates to polypeptides comprising a region which is capable of binding a target with high affinity, especially those comprising immunoglobulin chain variable domains (ICVD) as well as to constructs comprising said polypeptides and pharmaceutical compositions comprising such polypeptides and constructs. The polypeptides, constructs and pharmaceutical compositions of the invention are all suitable for oral administration. The present invention also relates to methods of increasing the intestinal stability of a polypeptide comprising an immunoglobulin chain variable domain, methods of making a polypeptide comprising an immunoglobulin chain variable domain, and methods which utilise such polypeptides, constructs comprising such polypeptides, nucleic acids encoding such polypeptides, cDNA and vectors comprising nucleic acids encoding such polypeptides, host cells expressing or capable of expressing such polypeptides, pharmaceutical compositions comprising such polypeptides and to uses of such polypeptides.

BACKGROUND OF THE INVENTION

[0003] Pharmaceutical research and development is becoming increasingly focussed on biopharmaceuticals such as therapeutic polypeptides, including antibodies. Typically, therapeutic polypeptides are administered either directly or indirectly into the circulation, via a systemic route. However, many therapeutic polypeptides would ideally be delivered via the oral route. Delivering therapeutic polypeptides orally could provide the following advantages: (a) direct targeting to the gastrointestinal tract (GIT) for localised treatment of gastrointestinal diseases (Jones and Martino 2015 *Crit Rev Biotechnol* 20:1-15), (b) the risk of adverse immune reactions could be reduced due to the naturally immuno-tolerant nature of the GIT, ensuring the long-term safety of repeatedly ingesting therapeutic polypeptide materials, (c) without the stringent regulatory requirements of manufacturing injectable therapeutic polypeptides, production costs could be reduced and (d) higher levels of patient acceptance and long term compliance could be achieved (Shaji and Patole *Indian J Pharm Sci* 2008 70(3):269-277).

[0004] Many therapeutic polypeptides are, however, unstable in the intestinal tract and therefore the beneficial effect obtained from oral administration is generally limited (Bruno et al 2013 *Ther Deliv* 4(11):1443-1467). Consequently, oral dosage forms used for conventional small molecule drugs have been employed for oral polypeptide delivery. Various strategies currently under investigation include formulation vehicles, use of enzyme inhibitors, absorption enhancers and mucoadhesive polymers (Shaji and Patole, ibid).

[0005] Alternative strategies involving modifications to the therapeutic polypeptides themselves have also been employed, such as the introduction of (additional) cysteine bridges. Hussack et al 2011 *PLoS ONE* 6(11):e28218 describe the introduction of additional cysteine bridges into anti-TcdA VHHs. The effectiveness of these additional cysteine bridges on increasing proteolytic stability was highly dependent on the specific protease concerned and in some circumstances these additional cysteine bridges were detrimental to recombinant production levels. Similarly, Kim et al 2014 mAbs 6:1 219-235 engineered human VL domains with disulphide bridges, with mixed results.

[0006] In theory, one could consider substituting specific amino acids in a therapeutic polypeptide which are believed to be responsible for low intestinal stability of the therapeutic polypeptide, in order to enhance stability in the intestinal tract. However, in the context of immunoglobulin chain variable domains, single substitutions in amino acid sequence may detrimentally impact binding capability. This is particularly relevant to the complementarity determining regions (CDRs) of an immunoglobulin chain variable domain, which are responsible for binding target antigen. For example, regarding in particular CDR3 of a VHH, it is known that " . . . inasmuch as the CDR3 amino acids either are in direct contact with the antigen or maintain and influence the conformation of the CDR3 amino acids that directly contact the antigen, the CDR3 amino acids responsible for reduced stability cannot be replaced without serious loss of affinity." (Muyldermans *Annu Rev Biochem* 2013 82:775-797). This view is reinforced by, for example, the finding that substitutions to a VHH targetting *C. jejuni* flagella, including in particular an R to G substitution in CDR2, caused a large decrease in binding capability of the VHH (approaching control) (Hussack et al 2014 *Protein Engineering, Design & Selection* 27(6):191-198).

[0007] There is a long-felt need therefore for polypeptides which have increased intestinal stability, and for methods to increase the intestinal stability of such polypeptides.

[0008] Polypeptides of the present invention may, in at least some embodiments, have one or more of the following advantages compared to substances of the prior art:

[0009] (i) increased suitability for oral administration;

[0010] (ii) increased suitability for local delivery to the intestinal tract following oral administration;

[0011] (iii) increased intestinal stability whilst substantially maintaining binding affinity and/or potency;

[0012] (iv) increased stability in a model of the intestinal tract such as the Standard Trypsin Intestinal Tract Model, the Standard Mouse Small Intestinal Supernatant Intestinal Tract Model or the Standard Human Faecal Supernatant Intestinal Tract Model, whilst maintaining binding affinity and/or potency;

[0013] (v) increased stability in the presence of proteases, for example (a) in the presence of proteases found in the small and/or large intestine and/or IBD inflammatory proteases, for example trypsin, chymotrypsin, MMPs, cathepsin, enteropeptidase, host inflammatory proteases and/or (b) in the presence of proteases from gut commensal microflora and/or patho-